

AD \_\_\_\_\_

Award Number: DAMD17-97-1-7112

TITLE: Hereditary Breast Cancer: Mutations Within BRCA1 and  
BRCA2 with Phenotypic Responses

PRINCIPAL INVESTIGATOR: Henry Lynch, M.D.

CONTRACTING ORGANIZATION: Creighton University  
Omaha, Nebraska 68178

REPORT DATE: July 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20010419 080

# REPORT DOCUMENTATION PAGE

OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE July 2000		3. REPORT TYPE AND DATES COVERED Annual (1 Jul 99 - 30 Jun 00)	
4. TITLE AND SUBTITLE Hereditary Breast Cancer: Mutations Within BRCA1 and BRCA2 with Phenotypic Responses				5. FUNDING NUMBERS DAMD17-97-1-7112	
6. AUTHOR(S) Henry Lynch, M.D.					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Creighton University Omaha, Nebraska 68178  E-MAIL: htlynch@creighton.edu				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) To date we have seventy-three Hereditary Breast/Ovarian Cancer families with identified BRCA1 or BRCA2 genetic mutations, wherein 24 additional cases of slides and tissue blocks have been retrieved. There are now 108 BRCA1 and 37 BRCA2 tumor cases that have slides and paraffin blocks available for histopathology and marker analysis. Evidence continues to accumulate that BRCA1 and BRCA2 HBCs are different in many of their pathobiologic features. Each present at a significantly earlier mean age (fifth decade vs. seventh decade for nonHBC). Compared to BRCA2 HBC, BRCA1 cases 1) are more prevalently aneuploid, 2) are higher grade, related to their high nuclear grade and high proliferative rates as measured by mitotic count and DNA flow cytometric S phase fraction, 3) have more medullary group carcinomas (typical and atypical medullary, ductal with medullary features), 4) have less tubular-lobular group carcinomas (lobular, tubulolobular, tubular, cribriform, variants), 5) have lesser attendant ductal and lobular carcinoma in situ, and 6) have lesser expression of estrogen receptor and c-erbB2 oncogene protein. Lobular neoplasia (lobular carcinoma in situ and atypical lobular hyperplasia) accompanied nearly all invasive carcinomas in one large BRCA2 family. In conclusion, BRCA1 and BRCA2 HBCs have distinctive pathobiologic phenotypes.					
14. SUBJECT TERMS Breast Cancer				15. NUMBER OF PAGES 18	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited		

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

## FOREWORD


Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

\_\_\_ Where copyrighted material is quoted, permission has been obtained to use such material.

\_\_\_ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

\_\_\_ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

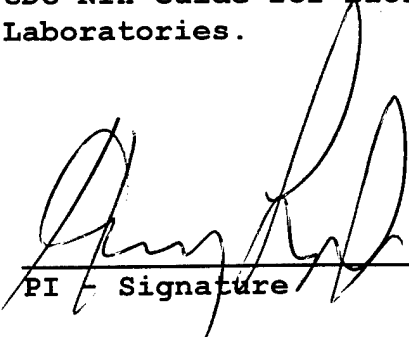
N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46. 

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

  
\_\_\_\_\_  
PI Signature      7/24/02  
Date

## Table of Contents

<b>Cover.....</b>	<b>1</b>
<b>SF 298.....</b>	<b>2</b>
<b>Foreword.....</b>	<b>3</b>
<b>Table of Contents.....</b>	<b>4</b>
<b>Introduction.....</b>	<b>5</b>
<b>Body.....</b>	<b>5</b>
<b>Key Research Accomplishments.....</b>	<b>7</b>
<b>Reportable Outcomes.....</b>	<b>7</b>
<b>Conclusions.....</b>	<b>8</b>
<b>References.....</b>	<b>11</b>
<b>Appendices.....</b>	<b>12</b>

## **INTRODUCTION**

The BRCA1 and BRCA2 cancer genes, isolated in 1994 and 1995, respectively, account for a substantial fraction of highly penetrant inherited breast cancer susceptibility. Each gene serves complex functions in the normal cell that include maintenance of the integrity of the genome, and for BRCA1, suppression of cell proliferation. Constitutive germline mutations in either can lead to the development of breast and ovarian neoplasia and might be expected to produce characteristic tumor phenotypes. Being able to identify and define these phenotypic changes will lead us to a better understanding of the etiology, pathogenesis, and survival of breast cancer patients who are part of BRCA1 and BRCA2 mutation positive families.

## **BODY**

To date, seventy-three Hereditary Breast Ovarian Cancer families have been ascertained for this study. Addresses and medical information has been updated throughout the study on individuals in these families.

Three hundred and forty-four cases have been identified as being eligible for this study with the addition of 116 over this past year. The status of these cases is presented in Table 1. Informed consents and permission forms to release clinical data, slides, and tissue blocks were sent to living subjects and to the next of kin of deceased subjects. To date, 240 informed consents have been returned.

One hundred forty-eight risk factor questionnaires have been completed by subjects and forwarded to Dr. Steven Narod's offices at the Women's College Hospital in Toronto, Canada for data entry and assessment of survival parameters in context with their staging classification.

Once a permission form was received from a subject a request for slides and tissues blocks was sent to the indicated treating hospital. Of the 230 requests sent to the hospitals, 54 requests were not answered by the hospital (subsequent requests were not answered also). Fifty-eight institutions reported the slides and blocks as being destroyed, missing, or not available. Many hospitals stated that the slides and blocks are destroyed after a set number of years. Obtaining the initial goal of 400 cases may not be realistic due to the lack of slides and tissue blocks being available. However, we will continue to strive to obtain as many cases as possible over this next year that has been granted for completion of the grant.

Over the past year we have collected 24 additional case samples of slides and tissue blocks. Please refer to Table 2 for the status of ascertained slides and tissue blocks. A random number was assigned to the sample and the H&E stained slides and corresponding blocks were sent to Dr. Norman Lehman in the Department of Pathology at Creighton University for selection of the best specimen. Once slides were selected, additional slides were cut from the accompanying blocks for the study and archive. DNA flow cytometry was performed, and tumors were classified histologically by two pathologists, Joseph Marcus, MD and David Page, MD, in a double-blind manner, all as before (4-6). Patients testing as mutation-negative in these families were excluded as were 2 cases each of BRCA1 and BRCA2 male breast cancer. If tumors were

not of a pure histologic type, they were classified as “variant” type or “possessing features” of the type if it occupied 50%-90% or 10%-50%, respectively, of the infiltrating tumor cross-section. Typical and atypical medullary carcinomas were classified by the criteria of Ridolfi et al (7). Immunohistochemical assays for estrogen receptor, progesterone receptor, and c-erbB-2 were performed according to manufacturer’s protocols on an automated immunostainer (Ventana Medical Systems, Tucson, AZ) using Ventana primary mouse monoclonal antibody clones 6F11, 1A6, and CB11, respectively. Statistical significances of differences were assessed by 2-tailed Fisher’s exact test for 2 x 2 contingency tables, and by Student t-test for means and standard deviations.

Since January 2000 four additional families have been identified with a BRCA1 or BRCA2 mutation. Individuals in these families who have been affected with breast cancer will be invited to participate in this study. We anticipate and look forward to the identification of new families throughout the year from Dr. Narod's laboratory. We anticipate the number of new families to increase since the recertification of Dr. Narod's laboratory has been completed. During the period of recertification, the testing was at a stand still for three months.

#### **KEY RESEARCH ACCOMPLISHMENTS & REPORTABLE OUTCOMES**

- 116 additional cases to collect slides and tissues blocks
- Tissue blocks and slide collection completed on 24 additional cases.
- 33 additional risk factor questionnaires were completed.
- 145 tumors analyzed by Dr. Joseph Marcus and Dr. David Page with reportable results.

## CONCLUSIONS & DISCUSSION

The families being followed at Creighton University represent one of the largest and longest-standing HBC and HBOC resources. From it Mulcahy and Platt reported an excess of medullary carcinomas in 1981 (1). We reported increased mitotic rates in 1988 (2) and suggested in 1994 that the proliferative phenotype was due to the BRCA1 subset (3). Our more extensive reports on the BRCA1 and BRCA2 phenotypes (4-6) were subsequently confirmed in most details by the Breast Cancer Linkage Consortium (8) and other groups, and our prediction (3) that BRCA1 mutations would confer a proliferative phenotype on the target cell was borne out in experiments *in vitro* and *in vivo* by Holt et al. in 1996 (9). The hyperproliferative BRCA1 phenotype suggests that BRCA1 breast cancers are highly evolved genetically, manifesting prevalent aneuploidy and chromosome alterations (4-6).

The proliferative characteristics of BRCA1 as compared with BRCA2 have come into sharper focus with the larger and genetically better defined data set presented here. They are dramatically illustrated by every measure – mitotic grade, mitotic rate, nuclear size, nuclear grade, and DNA S phase fraction. Our data also show a comparatively decreased estrogen receptor, progesterone receptor, and c-erbB2 oncoprotein expression in BRCA1 HBC, similar to the results of Johannsson et al (10). Despite so many dire prognostic markers (c-erbB2 excepted), BRCA1 breast cancers appear to have a prognosis no worse than non-HBC cases (11). Might this be related to the increased host lymphocytic and plasmacytic infiltration of these cancers and the greater representation of better-prognosis (but extremely proliferative) medullary types in



BRCA1 HBC? Medullary carcinomas characteristically produce an intense infiltration by host lymphocytes and plasma cells (7), and they have a greater expression of ICAM-1 cell adhesion molecules that partner with the LFA-1 ligand on lymphocytes (12). This suggests that a peculiar immune response may be driving the biologic behavior of BRCA1 HBCs. If the mechanism were better understood, immune intervention in the treatment of BRCA1 HBC could be envisioned.

In contrast, BRCA2 HBCs are more akin to usual non-hyperproliferating breast cancers, when this comparison is made (4-6), and they have lesser histologic evidence of immune modulation than BRCA1 HBCs. Unlike BRCA1 HBCs, there is not a deficit in “tubular-lobular group” carcinomas (lobular, tubulolobular, tubular, cribriform, and variants), and they may manifest a surfeit (4-6). BRCA2 HBCs are also attended by excess associated lobular neoplasia in some families (6). The BRCA2 pathophenotype may be more heterogeneous than the BRCA1 (6).

In summary, clinical features, the histopathology, DNA cytometry, and immunohistochemical markers for BRCA1 and BRCA2 carcinomas are shown in the tables. Significant differences ( $p < 0.05$ ) are in **bold**. The figures illustrate characteristic histopathologies in the two syndromes.

The data can be summarized as follows:

#### **BRCA1 vs. BRCA2 HBC**

- **Higher grade (nuclear, mitotic, total grades)**
- **More prevalent DNA aneuploidy**
- **Higher proliferation (high DNA aneuploid S phase fraction and mitotic rate)**

- **More medullary group carcinomas (typical and atypical medullary, ductal with medullary features)**
- **More tumor infiltration by mononuclear inflammatory cells**
- **Deficit of ductal and lobular in situ carcinoma**
- **Deficit of tubular-lobular group carcinomas (lobular, tubulolobular, tubular, cribriform, variants)**
- **Decreased expression of estrogen receptor and c-erbB2 oncogene protein**

When compared with non-HBCs (4,5), BRCA2 HBCs appear to be more similar to usual breast carcinomas. BRCA1 HBC appears to be the predominantly deviant phenotype. Please refer to Tables 2-6 for complete details.

## REFERENCES

1. Mulcahy GM, Platt R (1981). Pathologic aspects of familial carcinoma of the breast. In Genetics and breast cancer, HT Lynch, ed., Van Nostrand Reinhold, New York, 65-97.
2. Marcus J, Page D, Watson P, Conway T, Lynch H (1988). High mitotic grade in hereditary breast cancer. *Lab Invest* 58:60A.
3. Marcus JN, Watson P, Page DL, Lynch HT (1994). The pathology and heredity of breast cancer in younger women. *J Ntl Cancer Inst (Monogr)* 16:23-34.
4. Marcus JN, Watson P, Page DL, Narod SA, Lenoir GM, Tonin P, Linder-Stephenson L, Conway T, Lynch HT (1996). Hereditary breast cancer: Pathobiology, prognosis, and BRCA1 and BRCA2 gene linkage. *Cancer* 77:697-709.
5. Marcus JN, Page DL, Watson P, Narod SA, Lenoir GM, Lynch HT (1997). BRCA1 and BRCA2 hereditary breast cancer phenotypes. *Cancer* 80:534-556.
6. Marcus JN, Watson P, Page DL, Narod SA, Tonin P, Lenoir GM, Serova O, Lynch HT (1997). BRCA2 hereditary breast cancer pathophenotype. *Breast Cancer Res Treat* 44:275-277.
7. Ridolfi RL, Rosen PP, Port A, Kinne D, Mikð V (1977). Medullary carcinoma of the breast: A clinicopathologic study with 10 year follow-up *Cancer* 40:1365-1385.
8. Breast Cancer Linkage Consortium (1997). Pathology of familial breast cancer: Differences between breast cancers in carriers of BRCA1 or BRCA mutations and sporadic cases. *Lancet* 349:1505-1510.
9. Holt JT, Thompson ME, Szabo C, Robinson-Benion C, Arteaga CL, King M-C et al. Growth retardation and tumor inhibition by BRCA1 (1996). *Nat Genet* 12:298-302.
10. Johansson OT, Idvall I, Anderson C, Borg A, Barkardottir RB, Egilsson V, Olsson H (1997). Tumor biological features of BRCA1-induced breast and ovarian cancer. *Eur J Cancer* 33:362-371.
11. Watson P, Lynch HT, Marcus JN (1998). Prognosis of BRCA1 hereditary breast cancer. *Lancet* 351:304-305.
12. Bacus SS, Zelnick CR, Chin DM, Yarden Y, Kaminsky DB, Bennington J, Wen D, Marcus JN, Page DL (1994). Medullary carcinoma is associated with expression of intercellular adhesion molecule-1. *Am J Pathol* 145:1337-1348.

**Table 1: Status of Eligible Cases**

<b>Total Number of Eligible Cases</b>	<b>344</b>
<b>Unobtainable permission forms</b>	<b>114</b>
No response to letter	68
Treating hospital unknown	22
Lost to contact	15
Found to be deceased	5
Refused to participate	4
<b>Ascertained permission forms</b>	<b>230</b>
Hospital did not respond to request	54
Slides and Blocks no longer available	58

**Table 2: Status of Ascertained Slides and Tissue Blocks**

<b>Ascertained Slides and Tissue Blocks</b>	<b>118</b>
<b>Completed pathologic analysis*</b>	<b>54</b>
<b>Pending H&amp;E slides and DNA flow cytometry</b>	<b>43</b>
<b>Pending pathologic analysis</b>	<b>21</b>

\*Result analysis is based on 54 new cases ascertained during this grant period combined with previously collected data on slides and tissue blocks in the preliminary study.

<b>Table 3: Clinical Features</b>			
	BRCA1	BRCA2	p
Number of families	29	10	
Total number of tumors	108	37	
Bilateral cases	34 (31.5%)	8 (21.6%)	0.299
Male cases (excluded)	2	2	
<b>Mean age (yr) ∓ SD</b>	<b>42.9∓12.6</b>	<b>49.1∓12.3</b>	<b>0.011</b>
Mean tumor size (cm) ∓ SD	2.1∓1.3	1.8∓1.2	0.246
Lymph node positive cases	30/94 (31.9%)	12/24 (50.0%)	0.151

<b>Table 4: Histopathologic Classification</b>			
	BRCA1 n (%)	BRCA2 n (%)	p
<i>All Carcinomas</i>	<i>N=108</i>	<i>N=37</i>	
<b>Primary DCIS</b>	<b>2 (1.9)</b>	<b>5(13.5)</b>	<b>0.012</b>
Primary LCIS	0 (0.0)	0 (0.0)	1.000
<b>Any DCIS</b>	<b>31 (28.7)</b>	<b>20 (54.0)</b>	<b>0.009</b>
<b>Any LCIS</b>	<b>2 (1.9)</b>	<b>5 (13.5)</b>	<b>0.012</b>
<b>Any lobular neoplasia</b>	<b>3 (2.8)</b>	<b>11 (29.7)</b>	<b>&lt;0.0001</b>
Ductal	67 (62.0)	17 (45.9)	0.122
Medullary	16 (14.8)	1 (2.7)	0.072
Atypical medullary	16 (14.8)	3 (8.1)	0.403
Lobular	2 (1.9)	3 (8.1)	0.105
Lobular variant	4 (3.7)	4 (10.8)	0.203
Tubulolobular	0 (0.0)	2 (5.4)	0.064
Tubulolobular variant	1 (0.9)	0 (0.0)	1
Tubular variant	0 (0.0)	1 (2.7)	0.255
Cribriform	0 (0.0)	1 (2.7)	0.255
<i>Invasive Carcinomas</i>	<i>N=106</i>	<i>N=32</i>	
<b>Medullary group</b> (medullary, atypical medullary, ductal with medullary features)	<b>43 (40.6)</b>	<b>4 (12.5)</b>	<b>0.003</b>
<b>Tubular-lobular group</b> (lobular, tubulolobular, tubular, cribriform, variants)	<b>14 (13.2)</b>	<b>15 (46.9)</b>	<b>0.0001</b>

<b>Table 5: Histopathologic Features (Invasive Carcinomas)</b>			
	BRCA1 n (%)	BRCA2 n (%)	p
<b>Mitotic grade 3</b>	<b>43 (53.1)</b>	<b>6 (25.0)</b>	<b>0.020</b>
<b>Nuclear grade 3</b>	<b>45 (55.1)</b>	<b>2 (8.3)</b>	<b>&lt;0.0005</b>
Tubular grade 3	72 (88.9)	19 (79.2)	0.302
<b>Final grade 3 (ductals only)</b>	<b>39 (56.5)</b>	<b>4 (21.1)</b>	<b>0.009</b>
<b>Mononuclear infiltration absent</b>	<b>3/32 (9.4)</b>	<b>9/24 (37.5)</b>	<b>0.019</b>
	Mean $\forall$ SD n=32	Mean $\forall$ SD n=10	
Mitoses/2mm <sup>2</sup>	23.9 $\forall$ 25.7	11.7 $\forall$ 14.5	0.069
<b>Nuclear size (:)</b>	<b>13.3<math>\forall</math>3.1</b>	<b>10.3<math>\forall</math>1.8</b>	<b>0.001</b>



<b>Table 6: DNA Cytometry of the Invasive Carcinomas</b>			
	BRCA1 n (%)	BRCA n (%)	p
<b>Diploid</b>	<b>10 (14.9)</b>	<b>10 (50.0)</b>	<b>0.002</b>
<b>Aneuploid</b>	<b>57 (85.1)</b>	<b>10 (50.0)</b>	
	Mean $\nabla$ SD	Mean $\nabla$ SD	
DNA index	1.59 $\nabla$ 0.34 n=56	1.59 $\nabla$ 0.19 n=10	0.968
S phase fraction, % (diploids)	2.78 $\nabla$ 1.73 n=10	3.53 $\nabla$ 1.73 n=10	0.479
<b>S phase fraction, % (aneuploids)</b>	<b>15.77<math>\nabla</math>6.82</b> n=56	<b>7.36<math>\nabla</math>4.87</b> n=10	<b>&lt;0.0005</b>

<b>Table 7: Immunohistochemical Markers of the Invasive Carcinomas</b>			
	BRCA1 n (%)	BRCA2 n (%)	p
<b>Estrogen receptor</b>	<b>9/34 (26.5)</b>	<b>13/20 (65.0)</b>	<b>0.009</b>
Progesterone receptor	6/33 (18.2)	6/16 (37.5)	0.169
<b>c-erbB2</b>	<b>8/34 (23.5)</b>	<b>10/19 (52.6)</b>	<b>0.040</b>